The effect of anastomosis time on outcome in recipients of kidneys donated after brain death: a cohort study.

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Abbreviations: DGF, delayed graft function; OR, odds ratio; CI, confidence interval; SCD, standard criteria donation; ECD, expanded criteria donation; IFTA, interstitial fibrosis and tubular atrophy; CADI, chronic allograft damage index; PWV, pulse wave velocity; IMT, intima media thickness; BMI, body mass index; GFR, glomerular filtration rate; GLM, generalized linear models

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ABSTRACT

Whether warm ischemia during the time to complete the vascular anastomoses determines renal allograft function has not been investigated systematically. We investigated the effect of anastomosis time on allograft outcome in 669 first, single kidney transplantations from brain dead donors. Anastomosis time independently increased the risk of delayed graft function (odds ratio per minute (OR) 1.05, 95% confidence interval (CI) 1.02 – 1.07, $p < 0.001$) and independently impaired allograft function after transplantation ($p = 0.009$, mixed-models repeated-measures analysis). In a subgroup of transplant recipients, protocol-specified biopsies at 3 months (n=186), 1 year (n=189), and 2 years (n=153) were blindly reviewed. Prolonged anastomosis time independently increased the risk of interstitial fibrosis and tubular atrophy on these protocol-specified biopsies post-transplant ($p < 0.001$, generalized linear models). In conclusion, prolonged anastomosis time is not only detrimental for renal allograft outcome immediately after transplantation, also longer-term allograft function and histology are affected by the duration of this warm ischemia.

Introduction:

Ischemia-reperfusion injury is a major threat to the renal transplant. Prolonged cold ischemia time impairs allograft function, allograft histology, and survival (1-4). In addition, warm ischemia prior to organ procurement impacts organ viability (5-7). Furthermore, the organ is exposed to slow rewarming during transplantation, when the kidney is removed from the ice but not yet reperfused in the recipient. This period of warm ischemia is called the anastomosis time. It has been sporadically demonstrated that prolonged anastomosis time increased the risk of delayed graft function (DGF)(8, 9). Only recently, an independent effect of anastomosis time on patient survival has been demonstrated(10). However, the authors did not observe an independent effect on allograft survival and allograft survival was not censored for patient death. Moreover, no association with allograft function or histology was investigated. Thus, the precise impact of anastomosis time on the renal allograft remains unclear. Given the fact that kidney temperature increases logarithmically during anastomosis time (11), we hypothesized that anastomosis time associates with DGF, allograft histology, function and survival.

Methods:

Study population

All patients who received a first and single kidney transplant from a brain dead

donor at the University Hospitals Leuven, in Leuven, Belgium, between January 1, 2004 and December 31, 2012 were included in this cohort study. When donor warm ischemia time was reported to be present due to a delay in cold flush after cross clamping of the aorta, recipients were excluded from the study.

Clinical data

The clinical data of both donors and recipients were prospectively collected in electronic clinical patient charts, which were used for clinical patient management and directly linked to the database used in this study. Donation after brain death was categorized as standard (SCD) or expanded criteria donation (ECD). ECD was defined as any kidney procured from a donor aged ≥ 60 years or any donor aged 50-59 years with two of the following three criteria: cerebrovascular accident as cause of death, medical history of arterial hypertension and terminal creatinine > 1.5 mg/dL (or > 133 µmol/L) (12). Recipient BMI was measured on the day of transplantation. Anastomosis time and cold ischemia time were recorded during transplantation. Anastomosis time was defined as the time between the allograft leaving the ice and restoration of blood flow by opening of the vascular clamps in the recipient. During this time period, all kidneys were wrapped in an ice blanket and irrigated with ice water every 5 minutes (Figure S1).

All post-transplantation data were collected during routine clinical follow-up of the transplant recipients. DGF was defined as need for dialysis within the first seven days after transplantation. Allograft function was measured by the abbreviated

MDRD formula at 3 months, 1, 2 and 3 years post-transplant. We defined acute rejection as treatment for rejection within the first three months after transplantation. All-cause allograft failure was taken as time from transplantation to graft nephrectomy or return to dialysis, whichever was earlier, or to death of the patient with a functioning allograft. Survival of the patient was defined as time from transplantation until death. All patients provided written consent to use their clinical data for study purposes.

Allograft biopsies

In a subgroup of recipients, those transplanted between March 2004 and October 2007, percutaneous, ultrasound-guided core needle biopsies (2x14-gauge needle) of the allografts were performed at 3, 12, and 24 months after transplantation. All biopsy specimens were scored at the time by one pathologist according to the revised Banff criteria (13). Only biopsy specimens that were rescored again by the pathologist blinded for the clinical information were included in this study. The Chronic Allograft Damage Index (CADI) was calculated as the sum of histologic scores for tubular atrophy, interstitial fibrosis, interstitial inflammation, mesangial matrix increase, vascular intimal thickening and glomerulosclerosis (14).

Vascular calcifications and arterial stiffness

Arterial calcifications at the time of transplantation could potentially prolong the process to anastomose the vessels as well as negatively impact allograft

performance. Its presence was not prospectively recorded during surgery. Therefore, we included the results from aortic calcifications measured by means of lumbar X-ray in 253 randomly selected transplant recipients at the time of admission for kidney transplantation between October 2006 and March 2009. These measurements were performed as part of a study published by our group (15). From 250 of these 253 recipients, anastomosis time was recorded. Details on aortic calcification measurement are reported in the Supplemental Methods. In addition, arterial stiffness was measured during the second postoperative week in the same study cohort (15). Measurements were made in a quiet, temperature-controlled room after 10 minutes of supine rest. Blood pressure was measured with a validated oscillometric device (Omron 507, Omron Corp, Japan) in the non-fistula arm (n = 123). The mean of three measurements taken one minute apart was used. Pulse wave velocity (PWV) was measured with the SphygmoCor system, after abstinence from caffeine or smoking and after an overnight fast without intake of antihypertensive drugs (n = 116). Intima media thickness (IMT) was measured with Philips Envisor in a smaller subgroup of this study cohort (n = 73). Details on IMT measurements are reported in the Supplemental Methods.

Statistical analysis

Follow-up analysis of the study population ended July 1, 2014.

Discrete variables were reported as a percentage and continuous variables as mean ± standard deviation (SD) or as median and interquartile range (IQR) when data were skewed. Correlations were assessed by means of Pearson or Spearman analysis, as appropriate. The association of DGF with each variable (donor serum creatinine, donor age, ECD vs. SCD, recipient age, recipient BMI, total number of HLA mismatch, cold ischemia time, anastomosis time, year of transplant (defined in all statistical analyses as number of years between the day of transplantation and the day of data analysis, to consider time era)) was investigated using univariate logistic regression. For multivariate analysis, all variables associated with DGF in the univariate analysis with p value < 0.05 were entered. The association of donor last serum creatinine level, donor age, donor class (SCD vs. ECD), recipient age, recipient BMI, total number of HLA mismatch, cold ischemia time, year of transplant, DGF, and acute rejection during the first three months after transplantation with allograft function at 3 months, 1, 2 and 3 years post-transplant was investigated using univariate and multivariate linear regression models. To assess the association of these variables with the repeated measures of allograft function, we used a mixed-models repeated-measures analysis with an autoregressive covariance matrix, as based on the lowest Akaike's information criterion. Also, Kruskal-Wallis test was used to analyse the difference in estimated GFR according to anastomosis time, grouped as 35 minutes or shorter, between 36 and 45 minutes and longer than 45 minutes. Post hoc analysis was done by Dunn's multiple comparisons test. We used Kaplan-Meier curves to show allograft survival with associated p

values derived from the univariate log-rank test. Multivariate Cox proportional hazards analysis was used to investigate the effect of anastomosis time on survival corrected for independent variables significantly associated with survival in the univariate analysis. Histologic features were dichotomized as absent (if grade 0) or present (if grade 1 to 3) for logistic regression. Only variables significant in the univariate analyses were entered in the multivariate analyses. The effect on the repeatedly measured histologic features as a binary logistic response variable was measured by generalized linear models. The effect of anastomosis time on CADI was investigated with linear regression analysis and mixed-models repeated-measurements analysis with a compound symmetry covariance matrix, as based on the lowest Akaike's information criterion. Two-sided p values of less than 0.05 were considered to indicate statistical significance. We used SPSS software, version 22, for all statistical analyses and GraphPad Prism, version 6.0 (GraphPad Software), for data presentation.

Results

Patients

A total of 679 first, single kidney transplantations from brain dead donors were performed during the study period. Six recipients were excluded from the analysis because of the presence of donor warm ischemia time during organ procurement. From 669 patients anastomosis time was prospectively collected.

All reported data refer to these patients. Median follow-up after transplantation was 5.53 years (3.30-7.55). Demographic and clinical characteristics of donors and recipients are summarized in Table 1. The median anastomosis time was 34 minutes (30-40) (Figure S1).

Determinants of the anastomosis time

Anastomosis time correlated with recipient BMI ($r= 0.14$, $p<0.001$). There was a significant but very weak correlation between anastomosis time, donor age ($r= 0.09$, $p=0.03$), and cold ischemia time ($r=0.09$, $p=0.02$). No association with other recipient or donor characteristics could be observed (Table S1).

From 663 of 669 recipients, data on left or right donor kidney was available. There was no significant difference in anastomosis time between left and right donor kidneys (33 min (30-39) vs. 34 min (30-40), $p=0.14$). The presence of one or more arteries or veins did not influence anastomosis time (for arteries: 34 min (30-40) vs. 34 min (30-38); $p=0.52$, for veins: 34 min (30-40) vs. 35 min (30-41); $p=0.56$; for vessels in general: 34 min (30-40) vs. 34 min (30-38); $p=0.72$).

The presence and severity of aortic calcification of the recipient was not associated with anastomosis time ($n=250$, $p=0.44$ and $p=0.84$ respectively). In addition, anastomosis time did not correlate with hemodynamic parameters of vascular stiffness, or with IMT (Table S2).

Effect on DGF

17% (n = 121) of transplant recipients experienced DGF. Longer anastomosis time was the strongest factor associated with DGF ($p < 0.001$). Other factors associated with an increased risk of DGF were older donor age, ECD vs. SCD, older recipient age, higher recipient BMI, higher number of HLA mismatches, longer cold ischemia time and year of transplantation (Table S3). In multivariate logistic regression, longer anastomosis time remained the strongest independent factor associated with DGF (OR per minute 1.05, 95% CI 1.02-1.07, $p = 0.001$), together with ECD, recipient BMI, total number of HLA mismatches, longer cold ischemia time and time period of transplantation (Table 2). In kidneys from donors aged 65 years or older, no association between prolonged anastomosis time and the occurrence of DGF was observed (OR 1.03, 95% CI 0.97–1.11, $p = 0.32$).

Effect on acute rejection

There was no significant association between longer anastomosis time and the occurrence of acute rejection in the first 3 months after transplantation ($p = 0.94$).

Effect on allograft function

Longer anastomosis time associated significantly with lower estimated GFR at 3 months ($p < 0.001$, beta = -0.14), at 1 year ($p = 0.005$, beta = -0.12), at 2 years ($p < 0.001$, beta = -0.15), and at 3 years ($p = 0.02$, beta = -0.12). Other factors that associated with lower allograft function at all time points were older donor age, ECD vs. SCD, year of transplant and DGF (Table S4).

When anastomosis time was categorized in 10 minutes intervals (< 35 min, 35 - 44 minutes, ≥ 45 min), estimated GFR differed between the groups at all time points (Figure 1).

In the multivariate linear regression analysis, longer anastomosis time remained an independent factor associated with lower allograft function, even when corrected for DGF and acute rejection, up to 2 years after transplantation (Table 3). In these multivariate models, the association of anastomosis time with allograft function was lower due to the presence of donor age in the model, as there was a significant correlation between older donor age and longer anastomosis time (see above). This interaction was further explored by a sensitivity analysis, dividing patients according to donor age (< 65 years vs. ≥ 65 years). In recipients of kidneys from donors aged 65 years or more, no association between anastomosis time and estimated GFR could be observed, in contrast to recipients of kidneys of younger donor kidneys where the magnitude of the effect was higher compared to the total study cohort (Table 3, Figure 2). There was no interaction affect between donor age and anastomosis time (both as continuous variable) in the linear regression analyses of eGFR at 3 months ($p=0.88$), 1 year ($p=0.95$), 2 years ($p=0.15$) and 3 years ($p=0.73$). Thus, the effect of age is not of a simple linear form.

When recipients were grouped according to cold ischemia time (< 15 h vs. ≥ 15 h), the association between anastomosis time and estimated GFR was more pronounced in recipients with longer cold ischemia time (Table S5).

In the mixed-models repeated-measures analysis, anastomosis time was an independent significant determinant of the evolution of estimated GFR after transplantation (p = 0.009) (Table 3).

Effect on allograft survival

There was no significantly reduced overall and death-censored allograft survival in patients with anastomosis time of 45 minutes or longer (log rank p = 0.07 and p = 0.06, respectively). There was no effect on patient survival (p = 0.38) (Figure 3). Also in a multivariate Cox regression analysis, corrected for variables significantly associated with survival in a univariate analysis (donor age, donor criteria, total number of HLA mismatch), anastomosis time was not associated with death-censored allograft survival (p = 0.49).

Effect on graft histology

To investigate whether the association between longer anastomosis time and lower allograft function was reflected by chronic histological lesions of the graft, we included protocol-specified biopsies at 3, 12, and 24 months from our cohort that were blindly rescored in this study (186 at 3 months, 189 at 1 year, and 153 at 2 year). We focused on interstitial fibrosis and tubular atrophy (IFTA) as this reflects chronic allograft injury and is associated with cold ischemia time as well (4). In allografts with IFTA present in protocol-specified biopsies at 1 and 2 years after transplantation, anastomosis time during transplantation was longer (p = 0.03 and p = 0.002, respectively) (Figure 4). Other variables associated with

IFTA are presented in Table S6. When adjusted for these significant variables, anastomosis time remained independently associated with IFTA at 1 and 2 years after transplantation ($p = 0.04$, OR 1.040 (95% CI 1.002-1.08), and $p = 0.009$, OR 1.06 (95% CI 1.02-1.12), respectively) (Table S7). When substracting the role of its components, mainly interstitial fibrosis correlated with anastomosis time. Generalized linear models analysis demonstrated that anastomosis time was significantly associated with the presence of IFTA on serial protocol biopsies (univariate $p < 0.001$, adjusted for significant covariates $p < 0.001$) (Table S6). There was no significant and consistent correlation between any other histologic finding and anastomosis time (Table S8). Only in the protocol-specified biopsies one year after transplantation did CADI associate with anastomosis time ($p = 0.26$, $p = 0.04$, and $p = 0.11$ for 3 months, 1, and 2 years after transplantation respectively). The overall effect of anastomosis time on the CADI score in the unadjusted mixed-models repeated-measures analysis was also significant ($p = 0.04$).

Discussion

In recipients of kidneys from brain-dead donors, the time taken to complete the vascular anastomoses during transplantation associates independently with an increased risk of DGF, with IFTA on protocol-specified biopsies and with worse allograft function, up to three years after transplantation.

As far as we are aware, the present study is the first to investigate the effect of anastomosis time on DGF, allograft function, histology and survival. In our patient cohort every minute increase in anastomosis time was accompanied with a 5% increased risk of DGF, independently from other risk factors. In addition, prolonged anastomosis time had a detrimental effect on allograft histology and function up to 3 years after transplantation. Even after correction for DGF, the association with impaired allograft function remained up to 2 years post-transplant. We did not observe a significant reduction in death-censored allograft survival with prolonged anastomosis time. However, our study did not have the adequate power to definitely answer this important clinical question, which has to be addressed in future, larger cohorts. One could speculate that by affecting allograft function up to 3 years after transplantation longer-term function will be affected as well, as allograft function at 1 year is considered the best predictor of long term function (16). In concordance, a large study only recently revealed an effect of anastomosis time on patient and allograft survival. Although the effect on allograft survival did not remain when adjusted for other variables in this study, it did when only first transplantations were considered (10).

In contrast to previous studies (2-4), cold ischemia time had no effect on allograft function or histology in our study cohort. However, in a recent publication by Summers et al. duration of cold ischemia time was not associated with decreased allograft survival for kidneys from brain dead donors (17). Moreover,

data from the Collaborate Transplant Study show that cold ischemic time, when less than 18 h, has no association with graft survival (18). It could be hypothesized that the range of cold ischemia time in our study cohort prevented us to observe any long-term effect. The low incidence of DGF in this study is probably also related to the short average cold ischemia time (15 ± 4h) in our practice.

To our surprise, the effect of anastomosis time on allograft function was more pronounced in kidneys from younger donors. This contrasts the idea that the impact of prolonged ischemia is particularly detrimental in older allografts. However, the evidence behind this concept is based on animal studies (19), or based on studies in non-kidney organ transplantations that differ in methodology: these studies focused on recipient survival instead of long-term allograft function and histology (20, 21) or used a younger age as cutoff to categorize donor age (20). Moreover, animal studies on brain hypoxic-ischemic injury suggest that the susceptibility of the brain may not be linear with age, and that after a certain age the susceptibility to ischemia decreases (22). For example, after an hypoxic insult higher blood lactate and lower glucose was observed in arterial blood of younger animals compared to older (23). Another possibility is that the impact of donor age overwhelms the effect of anastomosis time in kidneys from older donors. In line with our observation is a study of 788 first cadaver kidney transplants by Asderakis et al., in which the effect of prolonged cold ischemia time on reduced allograft survival was more pronounced in younger kidneys (24). The analysis of

the effect of anastomosis time according to donor age needs further confirmation in a larger study cohort.

In addition, we observed that anastomosis time was in particular detrimental to allograft function when kidneys were previously preserved cold for 15 hours or more.

Our observations could have been confounded by other factors that prolong the process to complete the vascular anastomosis as well as negatively impact the allograft on the short or long term. BMI of the recipient could be an example, as anastomosis time is reported to be longer in obese patients and obesity could influence allograft function (10, 25). Indeed, the higher the recipient BMI, the longer it took to complete the vascular anastomosis. Therefore, multivariate analyses were adjusted for recipient BMI and still the effect of anastomosis time on the increased risk of DGF and diminished long-term allograft function remained.

In addition, vascular calcification on the recipient's vascular system could have complicated the anastomosis process. We did not find a correlation between arterial calcification and anastomosis time, suggesting that this did not represent a major bias in our findings, although it is still possible that external iliac arteries might differ in regard to the presence and extent of calcification compared to the aorta.

We hypothesized additionally that the shorter right renal vein might increase the length of anastomosis time. A recent large study compared left and right kidney recipients transplanted from the same deceased donor and observed a higher incidence of DGF and lower one-year allograft survival for right kidneys, primarily attributed to surgical complications (26). In our study anastomosis time did not differ significantly between right and left kidney recipients and the presence of one or more arteries or veins did not significantly prolong anastomosis time.

Our results point to the importance of protecting the kidney to warm ischemia, even when lasting only tens of minutes. Strategies to reduce the length of warm ischemia might be beneficial. Possible examples are ample exposure of the operative field and iliac vessels to facilitate the anastomosis, avoidance of large vascular patches that take longer to be sewed in, or perhaps even the use of an automatic anastomotic stapler device (27). One could also reduce the extent of warm ischemia by cooling the graft (28). Whether these strategies have a favorable impact on transplant outcome needs further investigation.

In conclusion, prolonged anastomosis time increased the risk of DGF, evoked interstitial fibrosis/tubular atrophy in allografts and deteriorated allograft function up to 3 years after transplantation in kidney transplant recipients from brain dead donors.

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Declaration of conflicts of interests:

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Table 1: Patient and transplantation characteristics (n = 669)

Donor age (years)	48 ± 15
Donor gender (M/F in percentage)	53/47
Donor last serum creatinine (µmol/L)	63 ± 23
Donor criteria (SCD vs. ECD)	470 (70%) vs. 199 (30%)
Recipient age (years)	55 ± 13
Recipient gender (M/F in percentage)	62/38
Recipient BMI (kg/ m^2)	25 ± 5
Total HLA mismatch	3 ± 1
Cold ischemia time (h)	15 ± 4
Anastomosis time (min)	34 (30 - 40)
DGF	112 (17%)
Acute rejection first 3 months	110 (16%)
3 month serum creatinine (mg/dl) (n = 646)	1.69 ± 0.65
3 month eGFR (mL/min/1.73m^2) (n = 646)	47 ± 17
1 year serum creatinine (mg/dl) (n = 598)	1.50 ± 0.51
1 year eGFR (mL/min/1.73m^2) (n = 598)	52 ± 18
2 year serum creatinine (mg/dl) (n = 512)	1.51 ± 0.58
2 year eGFR (mL/min/1.73m^2) (n = 512)	52 ± 18
3 year serum creatinine (mg/dl) (n = 373)	1.57 ± 0.73
3 year eGFR (mL/min/1.73m^2) (n = 373)	51 ± 20

Data are expressed as mean ± SD; median (interquartile range); or as number (percentage) where appropriate.

M, male; F, female; SCD, standard criteria donation; ECD, expanded criteria donation; h, hours; min, minutes; DGF, delayed graft function; eGFR, estimated glomerular filtration rate.

Table 2. Determinants of delayed graft function by multivariate logistic regression analysis.

	OR	95% CI	P value
Year of transplant (y)	0.88	0.80 – 0.97	**0.008**
Donor age (y)	0.99	0.97 – 1.01	0.44
Donor criteria (SCD vs ECD)	1.70	0.87 – 3.31	0.12
Recipient age (y)	1.02	0.99 – 1.04	0.13
Recipient BMI (kg/m^2)	1.06	1.01 - 1.11	**0.015**
Total number of HLA mismatch	1.13	0.96 – 1.35	0.15
Cold ischemia time (h)	1.07	1.02 - 1.13	**0.006**
Anastomosis time (min)	1.05	1.02 - 1.07	**0.001**

A $p < 0.05$ is considered significant (values in bold).
SCD, standard criteria donation; ECD, expanded criteria donation; OR, odds ratio; 95% CI, 95% confidence interval.

Table 3: Multivariate analysis of determinants of allograft function at 3 months, 1, 2 and 3 years after transplantation, and in general over time.

		3 Mo after Transplantation*		1 Y after Transplantation*		2 Y after Transplantation*		3 Y after Transplantation*		Over time°
		Beta	p value	Beta	p value	Beta	p value	Beta	p value	p value
All patients	Anastomosis time (min)	- 0.11	**0.009**	- 0.09	**0.03**	- 0.11	**0.02**	- 0.09	0.11	**0.009**
	Cold ischemia time (h)	- 0.005	0.91	- 0.03	0.47	- 0.05	0.31	0.07	0.22	0.73
	Donor age	- 0.24	**<0.001**	- 0.25	**<0.001**	- 0.26	**<0.001**	- 0.39	**<0.001**	**<0.001**
Donor age < 65 years (n=580)	Anastomosis time (min)	- 0.15	**0.002**	- 0.15	**0.003**	- 0.18	**<0.001**	- 0.14	**0.03**	**<0.001**
	Cold ischemia time (h)	- 0.003	0.96	- 0.03	0.52	- 0.06	0.29	0.05	0.39	0.64
Donor age ≥ 65 years (n=89)	Anastomosis time (min)	0.06	0.62	0.18	0.20	0.27	0.12	0.23	0.35	0.25
	Cold ischemia time (h)	- 0.08	0.51	- 0.17	0.17	- 0.19	0.26	- 0.07	0.77	0.36

*Multivariate linear regression analysis or °multivariate mixed-models repeated-measurements analysis adjusted for year of transplant, donor criteria, last donor serum creatinine, recipient age, recipient BMI, total number of HLA mismatch, DGF and acute rejection during first 3 months after transplantation.
A $p < 0.05$ is considered significant (values in bold).

List of figures:

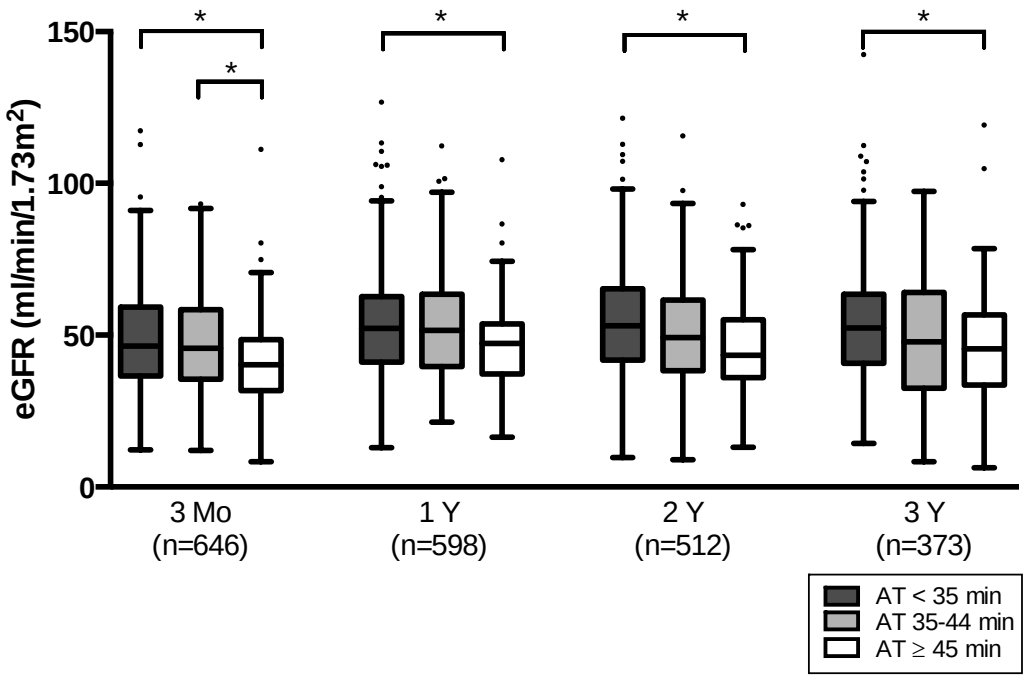

Figure 1: Estimated glomerular filtration rate (eGFR) at 3 months, 1 year, 2 years, 3 years after transplantation, according to anastomosis time, grouped as shorter than 35 minutes, between 35 and 44 minutes and 45 minutes or longer. At all time points, eGFR differs significantly (Kruskal Wallis test, p=0.002 at 3 months, p=0.05 at 1 year, p=0.004 at 2 years and p=0.02 at 3 years after transplantation). Post hoc analysis by Dunn's multiple comparisons test is significant (depicted as *) between recipients with anastomosis time of 45 minutes or longer compared to recipients with anastomosis time less than 35 minutes at all time points, and compared to recipients with anastomosis time between 35 and 44 minutes at 3 months after transplantation.

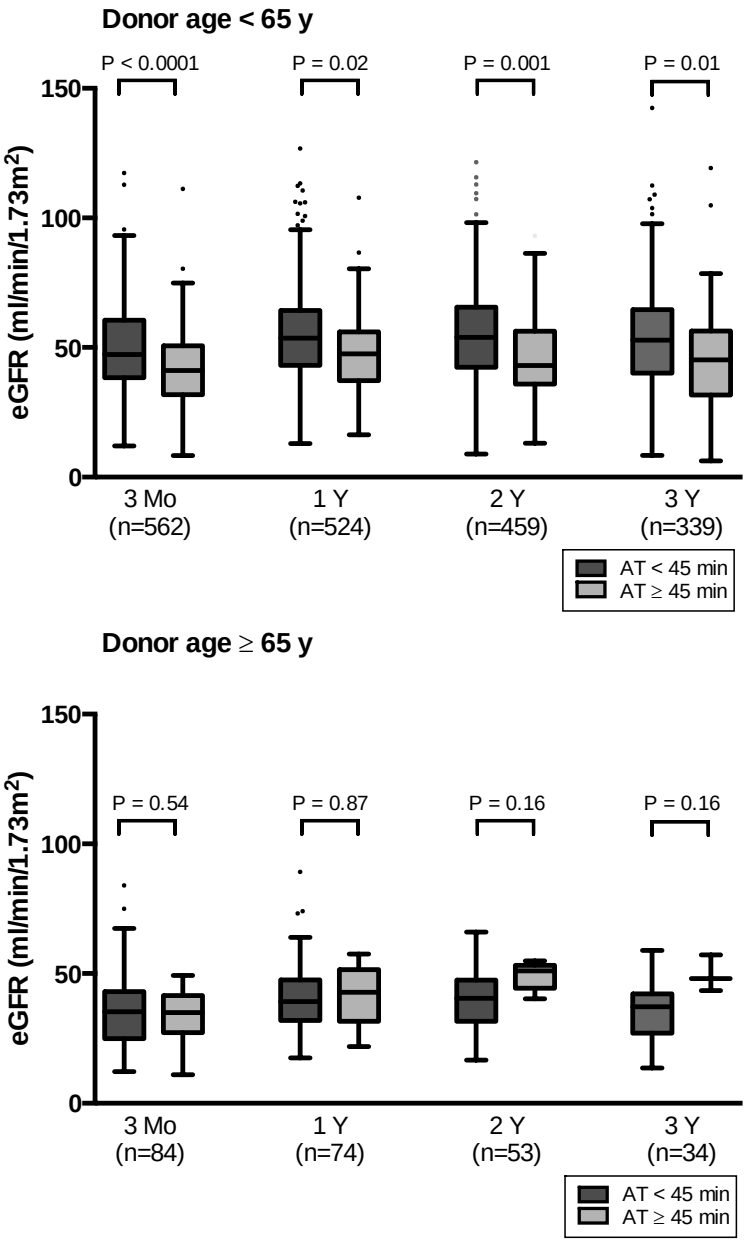

Figure 2: Estimated glomerular filtration rate (eGFR) at 3 months and 1, 2, 3 years after transplantation, according to anastomosis time (AT) and donor age. At all time points, eGFR differed significantly between high and low anastomosis time in recipients from donors aged less than 65 years, whereas no difference was observed in recipients from older donors. Data are presented in Tukey boxplots. P-value is measured by the Mann Whitney U test.

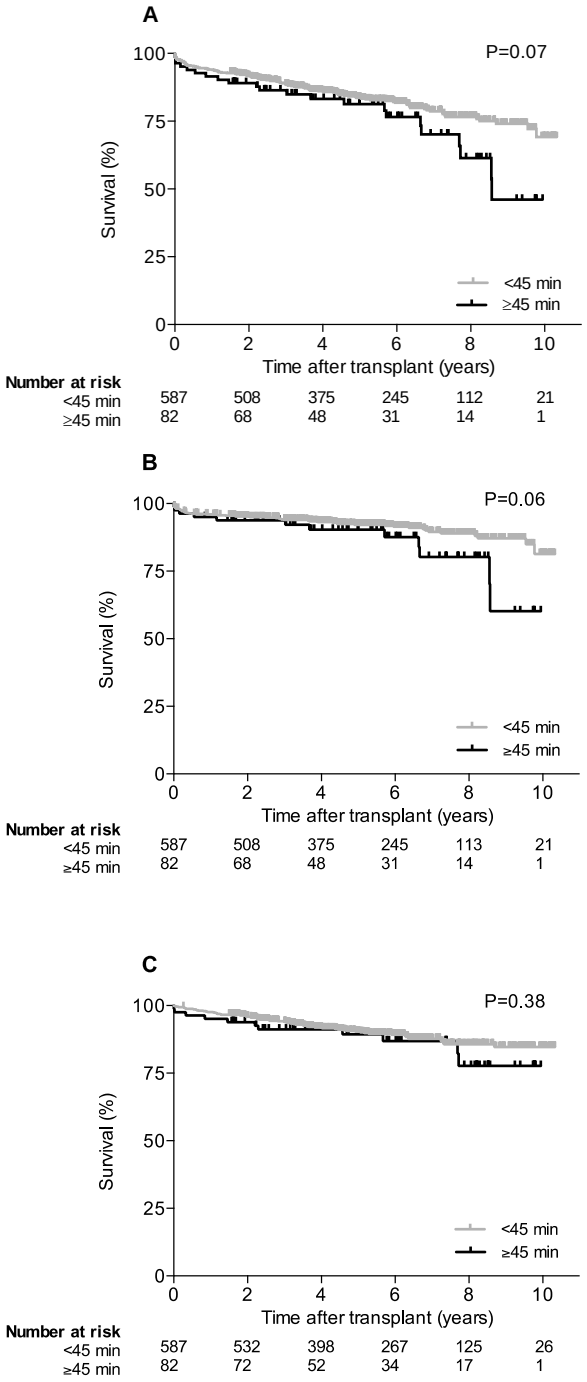

Figure 3: Kaplan Meier survival curves for allograft survival (Panel A), death-censored allograft survival (Panel B) and recipient survival (Panel C), stratified by anastomosis time. Data were censored at the time of data extraction from the clinical follow-up database (Jul 1, 2014). P values were calculated with the use of the log-rank test.

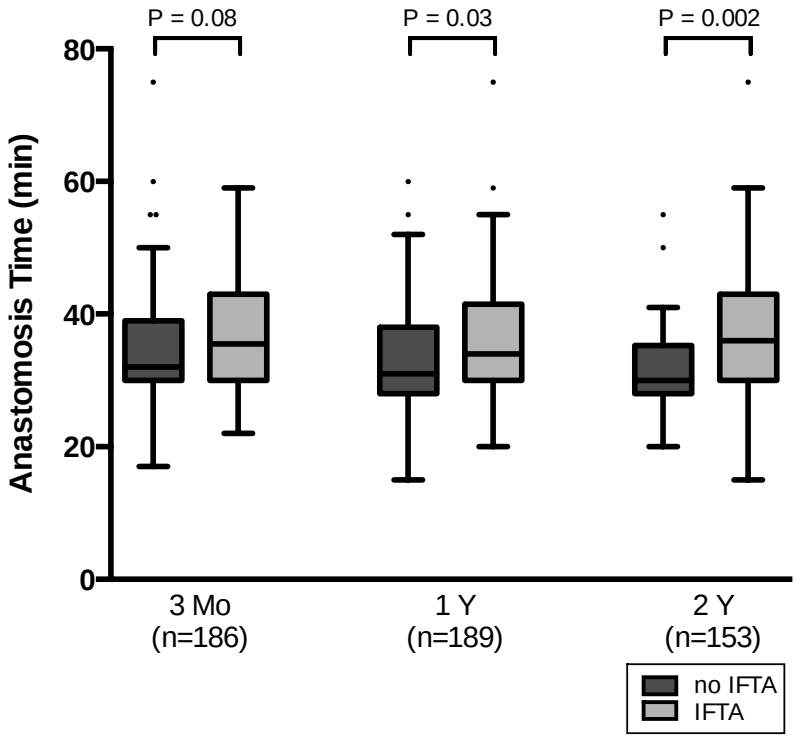

Figure 4: Anastomosis time according to the presence of interstitial fibrosis and tubular atrophy (IFTA) on protocol biopsies at 3 months, 1 year and 2 years after transplantation. Data are presented in Tukey boxplots. P value is measured by the Mann Whitney U test.